



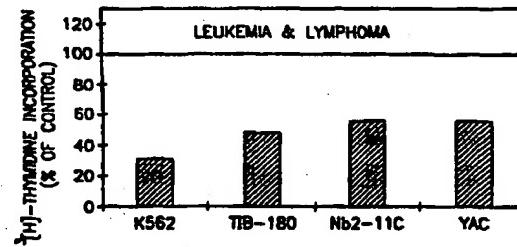
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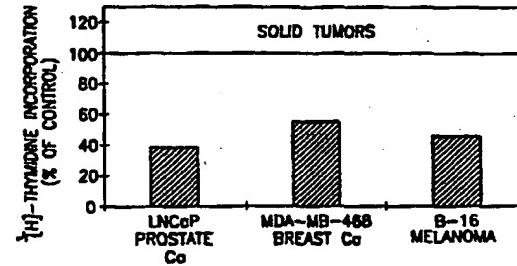
(54) Title: NUCLEOTIDE-COMPRISING COMPOSITION

(57) Abstract

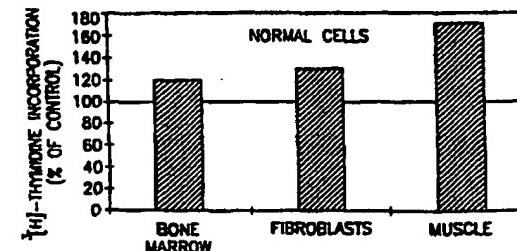
Adenosine and active agent which interact with the adenosine system are used to treat conditions of weakened, immune system, as an anti-cancer therapy and for improving the therapeutic index of a variety of therapeutic drugs.



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NUCLEOTIDE-COMPRISING COMPOSITION

FIELD OF THE INVENTION

The present invention concerns drugs for use in human therapy. More specifically, the present invention concerns pharmaceutical uses of adenosine as well as agents that interact with the adenosine system.

5

PRIOR ART

The following is a list of prior art references considered to be relevant as background to the invention:

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- 10 19. Jackson, R.C., Ross, D.A., Harkrader, R.J., Epstein, J., Biochemical approaches to enhancement of antitumor drug selectivity: selective protection of cells from 6-thioguanine and 6-mercaptopurine by adenosine, *Cancer Treatment Reports*, 64:1347-1353, 1980.
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- 25 22. Pines, M., Ashkenazi, A., Cohen-Shapnik, N., Binder, L. and Gertler, A., Inhibition of the proliferation of Nb2 cells by femtomolar concentrations of cholera toxin and partial reversal of the effects by 12-O-tetradecanoyl-phorbol-13-acetate, *J. of Cellular Biochem.*, 37:119-129, 1988.

The acknowledgement of these references herein will be made
30 by indicating their number from the above list, within brackets.

BACKGROUND OF THE INVENTION

Adenosine is a purine nucleoside which is known to act extracellularly to regulate different physiological processes through its binding to specific cell surface receptors (A₁ and A₂ receptors)^(1,2,3). The fact that adenosine exerts an extracellular effect was demonstrated, for example, by an increased effect of adenosine on cells when given together with dipyridamole, which inhibits uptake of adenosine by cells⁽⁴⁾.

Adenosine has been known to affect the cardiac rhythm and accordingly has been clinically used to protect the myocardium in various pathological situations^(5,6). In addition, adenosine is also known as having both vasoconstrictor effect on the kidney and a vasodilator effect in other 5 vascular beds. Adenosine is also known to have an effect on blood cells including: inhibition of platelet aggregation⁽⁷⁾; stimulation of red blood cell hematopoiesis through the production of erythropoietin⁽⁸⁾; exerting an anti-inflammatory effect manifested by inhibition of inflammatory cytokines^(9,10); and reduction of septic shock. Adenosine was also shown to 10 have a radioprotective effect when administered for a few minutes (about 15 mins.) prior to the radiotherapeutic treatment⁽¹¹⁾.

Adenosine was also known to exert an anti-proliferative effect on cancer cells. Bajaj *et al.*, 1983⁽¹²⁾ noted that adenosine and adenosine analogs have a toxic effect on lymphocytic leukemia cells, which was more 15 pronounced than the toxic effect on normal lymphocytes. Furthermore, Tey *et al.*, 1992⁽¹³⁾ showed that adenosine had an effect in modulating cell growth in human epidermoid carcinoma cells. In addition, it has been noted in the literature⁽¹⁴⁾ that parenterally administered adenine nucleotides (AMP, ADP and ATP) inhibit tumor development in mice⁽¹⁵⁾. ATP has been reported to 20 increase permeabilization of transformed cells^(16,17), but it should be noted that ATP exerts its effect through the cellular receptors, P2X, P2Y and P2Z⁽¹⁸⁾, which are different than that the A1 and A2 receptors through which adenosine exerts its regulatory effect.

White blood cells (leukocytes) consist of three basic groups of 25 cells: granulocytes, monocytes and lymphocytes. Many therapeutic drug treatments have undesired side effects manifested in reduction of leukocytes, and particularly reduction in the count and relative proportion of granulocytes (which are typically 70% of the white blood cells), and particularly of neutrophils, which typically constitute more than about 90% out of the

granulocytes. Examples of drugs which cause reduction in count of leukocytes, and particularly neutrophils, are cytotoxic drugs, e.g. such used in cancer chemotherapy, neuroleptic drugs, and others. The effect of reduction in white blood cell count is usually referred to in the art as "*leukopenia*" and
5 reduction in the count of neutrophils as "*neutropenia*". One major adverse effect of leukopenia, and particularly of neutropenia is an increase in susceptibility of individuals to opportunistic infectious diseases. In many cases, particularly in cancer chemotherapy, the patients very often die from such opportunistic infectious diseases (e.g. from lung infection) and not from
10 their primary disease, i.e. cancer. Drugs which can protect or inhibit the undesired toxic side effect of leukopenia, and particularly of neutropenia would thus be highly desirable. Jackson *et al.*⁽¹⁹⁾, reviewed by Gentile *et al.*⁽²⁰⁾, showed that the toxic effect of two purine analogs (6-thioguanine and 6-mercaptopurine) on lymphoblasts and fibroblasts can be inhibited by
15 adenosine. It was postulated that this effect is in view of the adenosine's inhibition of production of phosphoribosylpyrophosphate (PRPP). Jackson *et al.* point in their conclusion of their study to the possibility of developing cytotoxic drugs with a higher selectivity towards cancer cells, based on different enzyme patterns between tumor and normal cells. It should be noted,
20 that the work of Jackson *et al.* was conducted *in vitro* only, and when adenosine was tried *in vivo*, it was shown by Epstein *et al.*⁽²¹⁾ that *in vivo* protection towards toxic effect of purine analog can be achieved with adenosine levels of about 500 mg/kg body weight. It should, however, be noted that at such mega doses, adenosine would likely be toxic and have a
25 variety of side effects by itself.

GENERAL DESCRIPTION OF THE INVENTION

The present invention concerns a novel use of adenosine and various agents, including other nucleotides nucleoside derivatives, and

agents that interact with the adenosine system, that include: agonists and antagonists of adenosine receptors, inhibitors or blockers of adenosine transporters, as well as inhibitors of enzymes involved in adenosine metabolism, e.g. adenosine kinase inhibitor and adenosine diaminase 5 inhibitor.

In accordance with the present invention it was found that adenosine has an effect in inducing proliferation of bone marrow cells, resulting in increase in the number of leukocytes and particularly of neutrophils in the peripheral blood. It was furthermore found in accordance 10 with the invention that adenosine has a protective effect against some toxic effect of chemotherapeutic drugs, particularly protection against reduction in count of leukocytes, particularly neutrophils, which is otherwise caused by the chemotherapeutic drug. In addition, it was found in accordance with the invention that adenosine potentiates the specific anti-tumor activity of 15 chemotherapeutic drugs. Still further, it was found in accordance with the invention, that certain of the adenosine activities noted above can be modulated by various agents that interact with the adenosine system.

In *in vivo* studies in experimental animal it was shown that the overall effect of adenosine when administered together with a 20 chemotherapeutic drug is to increase the therapeutic index, namely, reducing toxic side effects and improving specific activity. In the following, the term "increase in therapeutic index" will be used to denote either improvement in the therapeutic effect of a drug, namely increasing its efficacy on a specific target cell, or reducing non desired toxic side effects manifested on cells other 25 than the target cells, or both.

In the following, the term "*target cells*" will be used to denote the target of said drug; the term "*non-target cells*" will be used to denote cells other than target cells on which the drug reserves a cytotoxic side effect.

The term "*anti-cancer chemotherapeutic drug*" will be used to denote a cytotoxic drug or a cocktail comprising a combination of two or more cytotoxic drugs given to an individual for the purpose of reducing the patient's tumor mass.

5 The term "*agents that interact with the adenosine system*" used above and which may be used further below, means to denote a variety of agents which interact with receptors, transporters or enzymes which regulate or mediate the adenosine interaction with cells. As known, extracellular adenosine can bind to a number of receptors on the cell membrane including, 10 among others, the A₁ and the A₂ receptors. Furthermore, cell membranes typically contain nucleoside transporters which can transport adenosine (as well as other nucleosides) into and out of the cell. Furthermore, in the extracellular medium surrounding the cells, as well as within the cells, there are various enzymes which can metabolize adenosine. The effect of 15 extracellularly applied adenosine on cells is a combination of these three different mechanisms, (namely binding to receptors, transport through nucleoside transporters and breakdown by enzymes. It was found in accordance with the invention that adenosine exerts its differential effect on bone marrow cells on the one hand and cancer cells on the other hand, 20 through interaction via these mechanisms (different ones in each case) and use of agents which interact through these mechanisms may thus have similar effects to that of adenosine or an effect in modulating the adenosine activity. Agents that interact with the adenosine system include receptor agonists and antagonists, adenosine diaminase or adenosine kinase inhibitors, transport 25 inhibitors, etc. In addition, as will no doubt be appreciated by the artisan, although the use of adenosine is preferred in accordance with the invention, other nucleosides as well as nucleoside derivatives may potentially be used to obtain qualitatively similar effects to that of adenosine. In the following, the invention will be described at times with particular reference to adenosine, it

being understood that the invention is not limited thereto but rather applies also to all the other active ingredients mentioned above.

In addition, as will be explained further below, the invention has several different aspects. One aspect is concerned with the induction of proliferation of bone marrow cells. Another aspect is concerned with inhibition of proliferation of tumor cells. A third aspect is concerned with the increase efficacy of some drugs, notably chemotherapeutic drugs, on target cells, particularly cancer cells. A fourth aspect is concerned with increasing therapeutic index of certain drugs, notably chemotherapeutic and neuroleptic drugs, by either increasing their specific effect on the target cell, by reducing their toxic side effects in reducing blood leukocyte count, or both. While adenosine is useful, and indeed the preferred active agent in all such aspects of the invention, other active ingredients which may be contemplated in accordance with the invention may not be readily utilized in all aspects of the invention and may thus be chosen for use in connection with one or more specific aspects only. For example, an adenosine receptor antagonist may be used in the bone marrow proliferation inducing aspect of the invention, as it was found to have such an activity. Against this, the adenosine receptor antagonist was found to have an effect in neutralizing adenosine's inhibitory effect on proliferation of tumor cells, and accordingly may not be used in the tumor proliferation inhibition aspect of the invention.

Active ingredients other than adenosine for use in each of the invention's therapeutic aspects, may be chosen based on simple screening using *in vitro* proliferation assays.

Many drugs have cytotoxic side effects on a variety of cells, particularly metabolically active and dividing cells. These include hematopoietic cells such as bone marrow cells, leukocytes, particularly neutrophils, as well as fibroblasts, cells of the digestive tract, and others. In the following, a reduction in leukocyte count or neutrophil count by cytotoxic

drugs, will be referred to herein, at times, as "*drug-induced leukopenia*" or "*drug-induced neutropenia*", respectively.

In the following, whenever mention is made to "*leukopenia*", it should be understood as referring particularly to "*neutropenia*" (drug-induced leukopenia is primarily manifested in reduction in the level of neutrophils).

Adenosine and some other of said active ingredients may be used in accordance with one aspect of the invention for inducing proliferation of bone marrow cells. This aspect of the invention is applicable in a variety of clinical situations. For example, adenosine and the other of said active ingredients may be used for treatment of patients having a disease or disorder caused by or associated with weakening of the immune system, e.g. in the case of inherited or acquired immune deficiency. Also certain autoimmune diseases are associated with an overall weakening of the immune system and said active ingredients may be used then as well. Another situation of a weakened immune system which may be treated in accordance with this aspect of the invention, is such which often occurs in advanced stages of cancer. This aspect of the invention is also useful in therapy in the case of leukopenia and particularly neutropenia occurring as a result of chemotherapeutic drug or neurleptic drug administration. The effect of adenosine or the other of said active ingredients is countering drug-induced leukopenia, can be manifested by more limited reduction in the amount (or count) of the leukocytes, as compared to the count without adenosine, or at times even an increase in the amount of the cells, even to levels above control.

In accordance with an additional aspect of the invention, adenosine, and some of said active ingredients, are used to inhibit proliferation of tumor cells, within the framework of anti-cancer therapy.

Furthermore, in accordance with another aspect of the invention, adenosine and some of said active ingredients may also be used to increase efficacy of cytotoxic drugs towards certain target cells, particularly of

anti-cancer chemotherapeutic drugs towards cancer cells. This increase in efficacy can be manifested by a more pronounced destruction of tumor cells at a given dose of the drug, or reduction in the required dose of the drug needed to achieve a certain therapeutic effect.

5 By a further of its aspects, adenosine as well as some of said active ingredients other than adenosine, are used for improving the therapeutic index of certain therapeutic drugs, particularly improving the ratio between a specific therapeutic effect of the chemotherapeutic drug or neuroleptic drug on its respective target cell versus toxic side effects of these drugs manifested
10 by leukopenia. In other words, the adenosine or some of the active ingredients other than the adenosine may give rise either to an increase in efficacy of a drug which may mean the ability to use a lower dose, to shorten the treatment period, etc., may give rise to reduction of the aforementioned toxic side effect, or both. The active ingredient in accordance with this aspect may be
15 administered in combination with the therapeutic drug, e.g. may be included together in one pharmaceutical composition with said therapeutic drug.

The present invention provides, for each of the abovementioned aspects, a method of therapeutic treatment comprising administering an effective amount of the respective active ingredient to an individual in need.
20 Still further provided for each of the aspects of the invention, is the use of the active ingredient for the purpose of preparing a pharmaceutical composition for the therapeutic treatments of diseases or conditions which are within the scope of the invention's various aspects, noted above. Furthermore, for each of these aspects, the present invention provides a pharmaceutical composition
25 for use in the treatment of a variety of conditions, diseases or disorders, which are within the scope of the above aspects, the composition comprising an effective amount of said active ingredient.

The term "*effective amount*" used above and below should be understood as meaning an amount of adenosine or another of said active

ingredients other than adenosine which is capable of achieving a desired therapeutic effect. The desired therapeutic effect depends on the type and mode of treatment. When, for example, said active ingredient is administered to counter drug-induced leukopenia, an effective amount of the active 5 ingredient may be an amount which protects the individual against the drug-induced reduction in the count of leukocytes, particularly neutrophils; an amount of the active ingredient which can give rise to an increase in an already decreased level of such cells, e.g. restore the level to a normal level or sometimes even above; etc. Where the active ingredient is administered in 10 order to potentiate the effect of an anti-cancer chemotherapeutic drug, an effective amount may be an amount which either increases the cancer specific toxicity of the chemotherapeutic treatment; an amount which is effective in reducing the amount of the chemotherapeutic drug or drug combination required to achieve a desired effect of the chemotherapeutic drug or drug 15 combination, i.e. reduction of the tumor load; etc. An example of an effective amount is a daily administration of adenosine of above about 10 µg./kg body weight, preferably at least about 25 µg/Kg body weight; and below about 50 mg/Kg body weight, preferably below about 10 mg/Kg body weight and most preferably below about 1 mg/Kg body weight. Typically, the amount of 20 adenosine administered will be within the range of 30-100 µg/kg body weight. Such an amount of adenosine is typically administered in a single daily dose although at times a daily dose may be divided into several doses administered throughout the day or at times several daily doses may be combined into a single dose to be given to the patient once every several days, 25 particularly if administered in a sustained release formulation.

By one embodiment of the invention, the composition, particularly where the active ingredient is adenosine composition is an oral composition. Such a composition may be provided as a liquid, e.g. a syrup; it may be provided as powder or lyophilisate for mixing with a palatable liquid

prior to administration; it may be provided in a dosage form, e.g. in the form of a capsule or a pill, etc.

By another embodiment, the composition is formulated for parenteral administration (intravenous, intramuscular, subcutaneous or 5 intraperitoneal administration). A composition in accordance with this embodiment, may be provided as a liquid mixture ready for use, or may also be provided as a powder or lyophilisate for mixing with saline or any other physiological liquid prior to use.

In accordance with a further embodiment, the adenosine 10 composition is provided as an inhalable composition in the form of a spray or aerosol.

In accordance with a still further embodiment, the composition is provided in the form of a patch for transdermal administration.

In the case of the aspects of the invention concerned with 15 reducing toxic side effects or increasing therapeutic index of therapeutic drugs, the administration of said active ingredient may begin a period of time, several days, prior to treatment with the therapeutic drug, e.g. the chemotherapeutic drug or a neuroleptic drug, and may then be continued also throughout the period of administration of the drug. Such prior administration 20 may provide an extra protective effect, for example against the drug-induced leukopenia.

At times a pre-treatment with said active ingredient may be sufficient, and at other times said active ingredient may be administered once or several times during the period of administration of the therapeutic drug. 25 During a period of combined administration of the active ingredient and the therapeutic drug, there may be a variety of possible combined administration regimes, for example: daily administration of both daily administration of the therapeutic drug and several times daily administration of the active

ingredient; a repeated cycle of administration comprising administering the therapeutic drug on one day and then the active ingredient on another day; etc.

According to an embodiment of the invention, the said active ingredient may be combined into a single composition with the other drug, and such a combined composition also forms as aspect of the invention.

The invention will now be illustrated in a non-limiting manner, by the following examples with occasional reference to the annexed drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the effect of increasing adenosine concentrations on the proliferation of Nb2-11c lymphoma cells, measured by cell count and expressed as percent of control.

Fig. 2 shows the effect of 4 μM of adenosine on the proliferation of tumor and normal cells: Panel A shows the effect of adenosine on a number of leukemia and lymphoma cell lines; Panel B shows the effect of adenosine on a number of solid tumors; and Panel C shows the effect of adenosine on normal cells. Proliferation of cells was measured by a thymidine incorporation assay.

Fig. 3 shows the effect of different doses of adenosine on proliferation of bone marrow cells (measured by a thymidine incorporation assay).

Fig. 4 shows the effect of theophylline in neutralizing the inhibitory effect of adenosine on tumor cell proliferation.

Fig. 5 shows the effect of adenosine receptor antagonists on proliferation of bone marrow cells (dark-colored columns show proliferation of bone marrow without adenosine; light-colored columns show the effect of proliferation in the presence of adenosine).

Fig. 6 shows the *in vivo* anti-tumor effect of three different treatments: administration of adenosine, administration of a chemotherapeutic drug — cyclophosphamide and a combination of adenosine and the chemotherapeutic

drug. The effect was measured by counting the number of tumor foci in the lungs of the tested mice.

Fig. 7 shows the *in vitro* effect of adenosine on activity of human natural killer (NK) cells and IL-2 production by human peripheral mononuclear cells. (Control – dark columns; cells exposed to adenosine – light colored columns).

Fig. 8 shows the effect of *in vivo* administered adenosine on activity of NK cells. (Dark-colored columns – NK cells obtained from naive mice; light-colored columns – NK cells obtained from adenosine-treated mice).

10

DETAILED DESCRIPTION OF THE INVENTION

Example 1 *In vitro studies*

Material and Methods

a. Tumor Cells

15

Tumor cell lines from human (K562 myeloid leukemia, Tib-180 erythroleukemia, LNCaP prostate carcinoma and MDA-MB-468 breast adenocarcinoma), and murine (Yac lymphoma, B-16 melanoma) were used and were purchased from the American Type Tissue Culture Collection, Rockville. Nb2 11c rat lymphoma cells (Pines *et al.*⁽¹⁹⁾) were also used. The cells were routinely maintained in RPMI medium containing 10% fetal calf serum. Twice a week the cells were transferred to a freshly prepared medium.

20

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Normal Cells

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As a control for tumor proliferating cells, two normal proliferating cell types were used i.e., bone marrow cells derived from the femur of C57BL/6J mice; fibroblasts derived from primary cultures of newborn rat skeletal muscle cells. The cells were prepared as previously, (Djaldetti, M., Sredni, B., Zigelman, R., Verber, M.,

and Fishman, P. Muscle cells produce a low molecular weight factor with anti-cancer activity. *Clin. Exp. Metastasis*, **14**:189-196, 1996). Also used was the L-8 myoblast rat cell line which was purchased from the ATCC.

5

c. Cell proliferation assays

(i) **Cell count assay:**

The effect of adenosine on the proliferation of rat Nb2-11C lymphoma cells was monitored by cell counting. The Nb2-11C cells were synchronized in the G0/G1 phase prior to cultivation with the CM by transferring the cells to horse serum (Biological Industries, Beit Haemek, Israel) supplemented medium for overnight incubation. 1.2x10⁵ cells/ml were cultured in 24 well plates in 1 ml RPMI medium containing 5% horse serum and 20, 10, 8, 6, 4, 2 and 1 µM of adenosine (purchased from Sigma, USA) were added. Cell proliferation was initiated by addition of Human Growth Hormone (Biotechnology General, Rehovot, Israel) to a final concentration of 2 ng/ml. The cultures were incubated at 37°C at 5% CO₂ and counted in Coulter Counter 48 hours later. Inhibition of cell proliferation was calculated as follows:

$$I = \frac{C_s - C_{nh}}{C_{wh} - C_{nh}}$$

wherein I = degree of inhibition (%)
 C_s = cell count with adenosine
 C_{nh} = cell count without hormone and without adenosine
 C_{wh} = cell count with hormone but without adenosine.

5 (ii) [³H]-Thymidine incorporation assay:

0.9x10⁴/well of each tumor cell line or normal cells (bone marrow cells or fibroblasts) were incubated with RPMI medium containing 10% FCS and 2 µM adenosine in 96 microtiter plates for 48 hours. During the last 6 hours of incubation, each well was pulsed with 1 µCi [³H]-Thymidine. The cells were harvested and the [³H-Thymidine uptake was determined in an LKB liquid scintillation counter (LKB, Piscataway, NJ, USA).

10

Results

a. Effect of adenosine on tumor and normal cell proliferation

15 The growth of the Nb2-11c rat lymphoma cells (measured by cell count) was markedly inhibited following incubation with different concentrations of adenosine. As can be seen in Fig. 1, there is a dose dependent effect of adenosine on the proliferation of the Nb2-11c cells.

20 Adenosine in a concentration of 4µM induced proliferation inhibition in the leukemia, lymphoma and the solid tumor cell lines, while in the same time it stimulated the proliferation of the three tested normal cells (Fig. 2): all results shown as % of control)

Additional studies revealed a dose dependent stimulatory effect of adenosine on bone marrow cell proliferation (Fig. 3) with a peak activity at about 10 µM).

25 b. Theophylline (adenosine A1&A2 receptor antagonist)
Neutralizes the inhibitory effect of adenosine on tumor cell proliferation

30 Theophylline is an antagonist of both A1 and A2 adenosine receptors. Theophylline in a concentration of 0.1µM was added to a culture of K562 cells in the presence of 4µM adenosine. Culture

conditions were identical to those described above. As can be seen in Fig. 4, while adenosine inhibited tumor cell proliferation in the presence of theophylline, this inhibitory effect was neutralized.

- 5 c. Effect of Theophylline (adenosine A1 and A2 receptor antagonist), DPCPX (adenosine A1 receptor antagonist) and DPSPX (adenosine A2 receptor antagonist) on bone marrow cell proliferation

10 Proliferation of bone marrow cells in the presence of the three adenosine receptor antagonists in the absence and in the presence of adenosine was tested. The concentration of Theophylline was 0.1 μ M; and that of DPCPX and DPSPX was 0.01 μ M. The concentration of adenosine was 10 μ M.

15 The results shown in Fig. 5 show that all these receptor antagonists caused a small increase in proliferation of bone marrow cells, while a much higher effect on increase of bone marrow cell proliferation was observed in the presence of adenosine. These results demonstrate that the stearatory effect of adenosine on bone marrow cells is likely through a mechanism not dependent on the adenosine receptors. In addition, although not wishing to be bound by theory, it is likely that the increase in proliferation obtained in the presence of the adenosine receptor antagonists is a result of freeing a larger amount of adenosine naturally present in the extracellular environment e.g. adenosine secreted by cells from internal pools, to act through the proliferation stimulation pathways.

20

25

Example 2 *In vivo studies***Material and Methods**

40 C57BL6/J mice were divided into 4 groups each of which were treated (by intraperitoneal injection) according to one of the following protocols:

- 5 1. control group: 6 days of treatment by daily injection of 1 ml saline.
- 10 2. adenosine and chemotherapy group: daily injection of a 1 ml 5 mM adenosine solution for 5 consecutive days. On day 6 the mice were injected intraperitonealy with cyclophosphamide.
- 15 3. adenosine group: daily injection of a 1 ml 5 mM adenosine solution for 5 consecutive days. On day 6 the mice were injected intraperitonealy with 1 ml saline.
4. chemotherapy group: daily injection of 1 ml saline for 5 consecutive days. On day 6 the mice were injected intraperitonealy with cyclophosphamide.

Five mice of each group were sacrificed 25 hrs and 96 hrs following the treatments and two parameters were evaluated:

- 20 1. Complete blood count cell.
2. Proliferation capacity of bone marrow: bone marrow was aspirated from the femur and pooled for each tested group. The cells were separated, counted and the proliferative capacity was evaluated by the [³H]-Thymidine incorporation assay as described above.

25 Results**Adenosine has a chemoprotective effect**

Following treatment with cyclophosphamide the number of white blood cell dropped after 24 hrs as is shown in Table 1, below. In the group which was treated 5 days with adenosine prior to the cyclophosphamide

administration, the number of white blood cells was even higher than that of the cyclophosphamide treated group.

Table 1

5

Group	WBC count x 10⁶/ml	
	24 hours	96 hours
control	6.0 ± 0.75	6.0 ± 0.75
adenosine	9.37 ± 0.26	6.8 ± 0.73
cyclophosphamide	3.8 ± 0.18	3.62 ± 0.17
adenosine + cyclophosphamide	6.4 ± 0.58	7.32 ± 0.88

10

The protective effect of adenosine was also demonstrated when testing bone marrow cell proliferation, as can be seen in Table 2 below. It can be seen that adenosine increased the proliferating capacity of the bone marrow even above control, even in the presence of the chemotherapeutic drug.

Table 2

	Bone Marrow Cell Proliferation (CPM) after 24 hours
Control	603.6 ± 23.7
Adenosine	1480.0 ± 68.5
Cyclophosphamide	434.5 ± 52.2
Adenosine + cyclophosphamide	1072.6 ± 85.3

15

Example 3 *In vivo* studies**Materials and Methods**

5 40 C57BL6/J were divided into 4 groups, each of which was treated (by intraperitoneal injection) according to one of the following protocols:

1. Control group: 6 days of treatment by daily injection of 1 ml saline.
- 10 2. Chemotherapy group: daily injection of 1 ml saline for 5 consecutive days. On day 6 the mice were injected intraperitoneally with cyclophosphamide.
- 15 3. Adenosine group: daily injection of a 1 ml group 5 mM adenosine solution for 5 consecutive days. On day 6 the mice were injected intraperitoneally with 1 ml saline.
- 20 4. Adenosine and chemotherapy group: daily injection of a 1 ml 5 mM adenosine solution for 5 consecutive days.

The following parameters were evaluated for each group:

- i. Red blood cell (RBC) count.
- ii. Hemoglobin (HGB) level.
- 20 iii. White blood cell count.
- iv. Proportion of the three major groups of white blood cells: Lymphocytes (LYM), Monocytes (MID) and Granulocytes (GRAN).

Results

25 The results (mean \pm S.E.) for each of the different groups is shown in the following Table 3.

Table 3

Group	RBC ¹	HGB ²	% LYM ⁴	% MID ⁴	% GRAN ⁴
Control	8.51±0.17	14.06±0.28	74.22±3.36	10.46±1.07	15.10±2.72
CHEMO	8.65±0.18	14.88±8.32	81.94±2.49	7.78±0.92	10.28±1.59
AD(w)	8.44±0.16	14.03±0.20	65.57±3.35	13.4±0.70	21.03±2.75
AD(w)+CH	8.17±0.28	13.06±0.46	75.1±3.89	9.00±0.86	15.84±3.08

1 Red blood cell counts - $10^6/ \text{ l}$

5 2 Hemoglobin count - in gram/100 ml

3 % out of the number of white blood cells

The above results demonstrate that while the chemotherapeutic
10 drug had almost no effect on the red blood cell count and on the level of hemoglobin, it caused a reduction in the amount of white blood cells. As can be seen, the white blood cell count was boosted to levels above control after administration of adenosine and when adenosine was administered together with the chemotherapeutic drug, the white blood cell count remained at levels
15 similar to control.

The above results demonstrate that while the chemotherapeutic drug had almost no effect on the red blood cell count and on the level of hemoglobin, it caused a reduction in the amount of white blood cells. As can be seen, the white blood cell count was boosted to levels above control after administration of adenosine and when adenosine was administered together with the chemotherapeutic drug, the white blood cell count remained at levels
20 similar to control.

While the chemotherapeutic drugs have a toxic effect and thus give rise to reduction in count of all white blood cells, this effect is more dominant on granulocytes. This can be seen by the fact that following administration of the chemotherapeutic drug the relative proportion of granulocytes decreases while the relative proportion of lymphocytes

decreases. This effect is reversed upon administration of adenosine, namely, adenosine has a more pronounced effect in causing proliferation of granulocytes in various other types of white blood cells.

5 Example 3 Additional *in vivo* studies

In a large number of *in vivo* studies conducted using similar protocols, the adenosine dosage which was used was reduced to about 54 µg/kg body weight per day, with a similar effect of adenosine, in toxic effect of the chemotherapeutic drug on the count of leukocytes and peripheral blood granulocytes. Additionally, in further experiments conducted in accordance with the invention it was found that in order to exert its protective effect, it is sufficient to administer the adenosine for several days prior to the treatment with the chemotherapeutic drug and this effect does not require continued administration of adenosine during the course of treatment with a chemotherapeutic drug.

Example 4 *In vivo* studies

Materials and Methods

B-16-F10 melanoma cells (2×10^5) were intravenously injected to 40 C57BL6/J mice. The mice were divided into 4 groups each of 10 mice which were treated by intraperitoneal administration of one of the following:

1. Control group: daily administration of 1 ml saline, per mouse from day of tumor inoculation until the mice were sacrificed.
2. Adenosine group: daily administration of 1 ml of a 5 mM adenosine solution per mouse from day of tumor inoculation until the mice were sacrificed.
3. Chemotherapy group: one injection of cyclophosphamide 24 hrs after inoculation of tumor cells and daily administration of 1 ml of

saline per mouse from day of tumor inoculation until the mice were sacrificed.

4. Chemotherapy and adenosine: one injection of cyclophosphamide 24 hrs after inoculation of tumor and daily administration of 1 ml of 5 mM per mouse from day of tumor inoculation until the mice were sacrificed.

Results

Following 18 days the mice were sacrificed and melanoma tumor foci were counted in the lung. The results are summarized in Table 4.

Table 4

Group	No. of foci
control	7.6 ± 2.68
adenosine	6.11 ± 2.74
chemotherapy	4.16 ± 1.42
adenosine + chemotherapy	3.0 ± 1.16

15

The above result demonstrates the adjunctive effect of adenosine in increasing potency of the anti-tumor effect of the chemotherapeutic drug.

20

In another experiment, a similar protocol was followed, with a lower dosage of adenosine (oral administration of 0.5 ml of a 10 µM adenosine solution, a dose equal to about 54 µg/kg body weight of adenosine). The results are shown in Fig. 6. As can be seen, here again, adenosine synergized with the chemotherapeutic drug to reduce the tumor low.

Example 5 *In vivo* studies**Materials and Methods**

50 C57BL6/J mice were divided into 5 groups of 10 mice each, which were treated by intraperitoneal injection with one of the following treatments:

1. Control group: 6 days of treatment by daily injection of 1 ml saline.
2. Adenosine group I: daily injection of 1 ml 5 M adenosine solution for 6 days (a dose of about 5 μ M/kg body weight).
- 10 3. Adenosine group II: daily injection of 1 ml 10 M adenosine solution for 6 days (a dose of about 1.08 μ g/kg body weight).
4. Chemotherapy group: daily injection of 1 ml saline for 5 consecutive days. On day 6 the mice were injected intraperitoneally with cyclophosphamide.
- 15 5. Adenosine and chemotherapy group: daily injection of 1 ml 10 M adenosine solution for 5 consecutive days. On day 6 the mice were injected intraperitoneally with cyclophosphamide.

Results

20 The total white blood cell count and the percentage of granulocytes, in each of the groups, is shown in the following Table 5.

Table 5

Group	WBC (count x 10 ³ / l)	GRAN (%)
Control	7.85	8.70
5 µM adenosine	9.50	9.00
10 µM adenosine	7.56	10.76
Chemotherapy	5.00	7.70
10 µM adenosine + chemotherapy	6.35	11.41

5 The above results show that adenosine at both concentrations had no effect in increasing the percentage of granulocytes in the white blood cell population. In addition, while the chemotherapy caused a decreased in the number of white blood cells and in the relative proportion of granulocytes, the adenosine was capable of restoring the total white blood cell count, 10 although not to full control levels, but more importantly, notwithstanding the chemotherapy, it yielded a very large increase in the relative proportion of granulocytes.

Example 6 Adenosine exerts an immunomodulator, effect

15 a. *In vivo* effect on NK activity and IL-12 secretion

Mononuclear cells (MNC) were fractionated from heparinized blood of 10 healthy volunteers using Ficoll-Hypaque gradient. 5×10^6 mononuclear cells/ml were incubated with 4µM adenosine and RPMI containing 10% FCS with and without 0.0075% of the mitogen 20 Staphylococcus Aureous Crown (SAC, Calbiochem Pansorbin) for 18 hours. At the end of the incubation period the supernatant was collected, centrifuged and filtered through 0.22µ sterile filter and kept at -70°C until

assayed. The level of IL-12 in the supernatants was analyzed using a commercial kit of R&D System.

The effect of adenosine on the activity of human peripheral blood NK cells was assayed by a standard 4h ^{51}Cr -release assay using K562 leukemia cells as targets. Peripheral blood mononuclear cells were separated from 10 healthy volunteers using Ficoll-Hypaque gradient. Cells were cultured at a concentration of 5×10^5 cells/well in 96 well round bottom plates , and used as the effector (E) cells. The cells were preincubated with $4\mu\text{M}$ adenosine in RPMI containing 5% FCS for 18 hours. K562 cells were used as the targets (T) and were labled with $100\mu\text{ci}$ of $\text{Na}_2[^{51}\text{Cr}]\text{O}_4$ at 37°C for 1h. After extensive washing to remove the excess Cr, cells (1×10^4) were resuspended and mixed with the effector cells at the E:T ratio of 1:50 in a volume of 200 μl using triplicate assays. After 4h of incubation at 37°C in 5% CO_2 , plates were centrifuged, and the supernatants were counted in a gamma counter (LKB).

NK cytotoxicity was calculated using the following equation:

$$20 \quad \% \text{ lysis} = \frac{\text{cpm experiment} - \text{cpm spontaneous}}{\text{cpm maximal} - \text{cpm spontaneous}} \times 10$$

where cpm spontaneous and cpm maximal were determined by measuring cpm of the supernatants of the target cells alone in the presence of assay medium or in the presence of 1% SDS, respectively. Spontaneous release was below 8% of the maximal release throughout this experiment.

Results:

Adenosine stimulated the production of IL-12 by human peripheral blood mononuclear cells in the presence of SAC mitogen (Fig. 7). In another set of experiments adenosine induced IL-12 production without the presence of

mitogen in the culture system (19% stimulation of IL-12 production). NK activity was also stimulated by 32% following the addition of adenosine to the culture system. It may be concluded that one of the potential mechanism through which adenosine stimulates NK activity is the induction of IL-12 production which is known as a activator of NK cells.

5 **b. In Vivo Effect of Adenosine on NK activity of mice splenocytes**

ICR mice were treated for 5 consequential days with 10 μ M of adenosine. Three days later the mice were sacrificed, spleens were taken out
10 and mononuclear cells were separated using ficoll hypaque gradient. NK activity was measured as described above for the human assay excluding the use of YAC murine lymphoma cells as targets instead of the K562 cells.

Results:

15 NK cells derived from adenosine treated mice were more potent in lysing the Yac taget cells as was seen in the two ratios of effectors:targets assayed (Fig. 8).

The modulating affect of adenosine may explaine some of its *in vivo* anti-tumoral activity shown below.

CLAIMS:

1. A pharmaceutical composition for the treatment of an individual having a reduced level of leukocytes in the circulation, comprising 5 an effective amount of an active ingredient selected from the group consisting of:

adenosine;

an active agent other than adenosine selected from the group consisting of nucleosides, nucleoside derivatives and agents which interact with the 10 adenosine system, said active agent being biologically active in inducing proliferation of bone marrow cells; or

or a combination of these active agents;

or a combination of adenosine and said substances.

2. A pharmaceutical composition according to Claim 1, wherein 15 said active ingredient is adenosine.

3. A pharmaceutical composition according to Claim 1 or 2, for treating an individual with a weakened immune system.

4. A pharmaceutical composition according to Claim 3, for 20 treating an individual undergoing a drug treatment causing reduction in leukocyte levels.

5. A pharmaceutical composition according to Claim 4, wherein the drug is a chemotherapeutic or neuroleptic drug.

6. A pharmaceutical composition for anti-cancer treatment, comprising an effective amount of an active ingredient, being:

25 adenosine;

an active agent other than adenosine selected from the group consisting of nucleosides, nucleoside derivatives and agents active in the adenosine system, said active agent being biologically active in inhibiting proliferation of tumor cells; or

a combination of adenosine and said active agent.

7. A pharmaceutical composition according to Claim 6, herein
said active agent is adenosine.

8. A pharmaceutical composition according to Claim 6 or 7, for
5 administering in combination with an anti-cancer chemotherapeutic drug.

9. A pharmaceutical composition according to Claim 6 or 7,
comprising an anti-cancer chemotherapeutic drug.

10. A pharmaceutical composition according to Claim 8 or 9,
wherein the amount of the chemotherapeutic drug is lower than that used in an
anti-cancer treatment where the chemotherapeutic drug is administered to the
10 individual without said active ingredient.

11. A pharmaceutical composition for use in improving therapeutic
index of a therapeutic drug which acts on specific target cells and having toxic
side effects in causing reduction in leukocyte levels of a treated individual, the
15 composition comprising an effective amount of an active ingredient selected
from the group consisting of:

adenosine;

an active agent other than adenosine selected from the group consisting
of nucleosides, nucleoside derivatives and agents active in the adenosine
20 system, said active agent being biologically active in either increasing
efficacy of the therapeutic drug or inducing proliferation of bone marrow
cells; or a combination of these active agents; or

a combination of adenosine and said agent.

12. A pharmaceutical composition according to Claim 11, wherein
25 said active ingredient is adenosine.

13. A pharmaceutical composition according to Claim 11 or 12,
wherein said therapeutic drug is a neuroleptic or chemotherapeutic drug.

14. A pharmaceutical composition according to any one of
Claims 11-13, wherein the effect of said active ingredient is manifested by

either permitting an increased dose of said therapeutic drug, an increase of administration period of said therapeutic drug, a reduced amount of said therapeutic drug yielding a therapeutic effect comparable to that achieved without said active ingredient.

- 5 15. A pharmaceutical composition according to any one of
Claims 11-14, comprising also said therapeutic drug.

10 16. A method of treating an individual having a reduced level of
leukocytes in the circulation, comprising administering to the individual an
effective amount of an active ingredient selected from the group consisting of:
adenosine;
an active agent other than adenosine selected from the group consisting
of nucleosides, nucleoside derivatives and an agent active in the adenosine
system, said active agent being biologically active in inducing proliferation of
bone marrow cells, or a combination of these active agents; or
a combination of adenosine and said active agent.

15 17. A method according to Claim 16, wherein said active ingredient
is adenosine.

20 18. A method according to Claim 16 or 17, wherein the treated
individual has a weakened immune system.

25 19. A method according to Claim 18, wherein the treated individual
undergoes a drug treatment causing a reduction in leukocyte levels.

20 20. A method according to Claim 19, for the treatment of drug-
induced leukopenia.

25 21. A method according to Claim 19 or 20, wherein the drug is a
chemotherapeutic or a neuroleptic drug.

20 22. A method for treating an individual suffering from cancer
comprising administering to the individual an effective amount of an active
ingredient selected from the group consisting of:
adenosine;

an active agent other than adenosine selected from the group consisting of nucleosides, nucleoside derivatives and an agent active in the adenosine system, said active agent being biologically active in inhibiting proliferation of tumor cells; or

5 a combination of adenosine and said agent.

23. A method according to Claim 22, wherein said active ingredient is adenosine.

24. A method according to Claim 22 or 23, comprising administering said active ingredient to the individual in combination with an
10 anti-cancer chemotherapeutic drug.

25. A method according to Claim 24, wherein the amount of the chemotherapeutic drug is lower than that used in anti-cancer treatment involving administering said chemotherapeutic drug without said active ingredient.

15 26. A method for treating an individual in order to increase the therapeutic index of a therapeutic drug, comprising administering said therapeutic drug in combination with an active ingredient selected from the group consisting of:

adenosine;

20 an active agent other than adenosine selected from the group consisting of nucleosides, nucleoside derivatives and an agent active in the adenosine system, said active agent being biologically active in either increasing efficacy of the therapeutic drug or inducing proliferation of bone marrow cells; or

25 a combination of adenosine and said agent.

27. A method according to Claim 26, wherein said active ingredient is adenosine.

28. A method according to Claim 26 or 27, wherein said therapeutic drug is an anti-cancer chemotherapeutic drug.

29. A method according to Claim 26 or 27, wherein said therapeutic drug is a neuroleptic drug.

30. A method according to any one of Claims 26-29, wherein the increase in therapeutic index is manifested by:

5 increase efficacy of said therapeutic drug at the administered amount as compared to administration without said active ingredient,

reduced toxic side effects of said therapeutic drug, or

increase in the duration of administration of said therapeutic drug.

31. A method according to any one of Claims 26-30, wherein said 10 therapeutic drug has a toxic side effect manifested in reduction of the level of leukocytes.

32. Use of an active ingredient selected from the group consisting of:

adenosine;

15 an active agent other than adenosine selected from the group consisting of nucleosides, nucleoside derivatives and agents which interact with the adenosine system, said active agent being biologically active in inducing proliferation of bone marrow cells; or

or a combination of these active agents;

20 or a combination of adenosine and said substances for the preparation of a pharmaceutical composition for the treatment of an individual having a reduced level of leukocytes in the circulation.

33. Use of an active ingredient selected from the group consisting of:

25 adenosine;

an active agent other than adenosine selected from the group consisting of nucleosides, nucleoside derivatives and agents which interact with the adenosine system, said active agent being biologically active in inducing proliferation of bone marrow cells; or

or a combination of these active agents;
or a combination of adenosine and said substances for the preparation of a pharmaceutical composition for anti-cancer treatment.

34. Use of an active ingredient selected from the group consisting

5 of:

adenosine;

an active agent other than adenosine selected from the group consisting of nucleosides, nucleoside derivatives and agents which interact with the adenosine system, said active agent being biologically active in inducing proliferation of bone marrow cells; or

or a combination of these active agents;

or a combination of adenosine and said substances for the preparation of a pharmaceutical composition for use in improving therapeutic index of a therapeutic drug which acts on specific target cells and having toxic side effects in causing reduction in leukocyte levels of a treated individual.

35. Use according to any one of Claims 32-34, wherein said active ingredient is adenosine.

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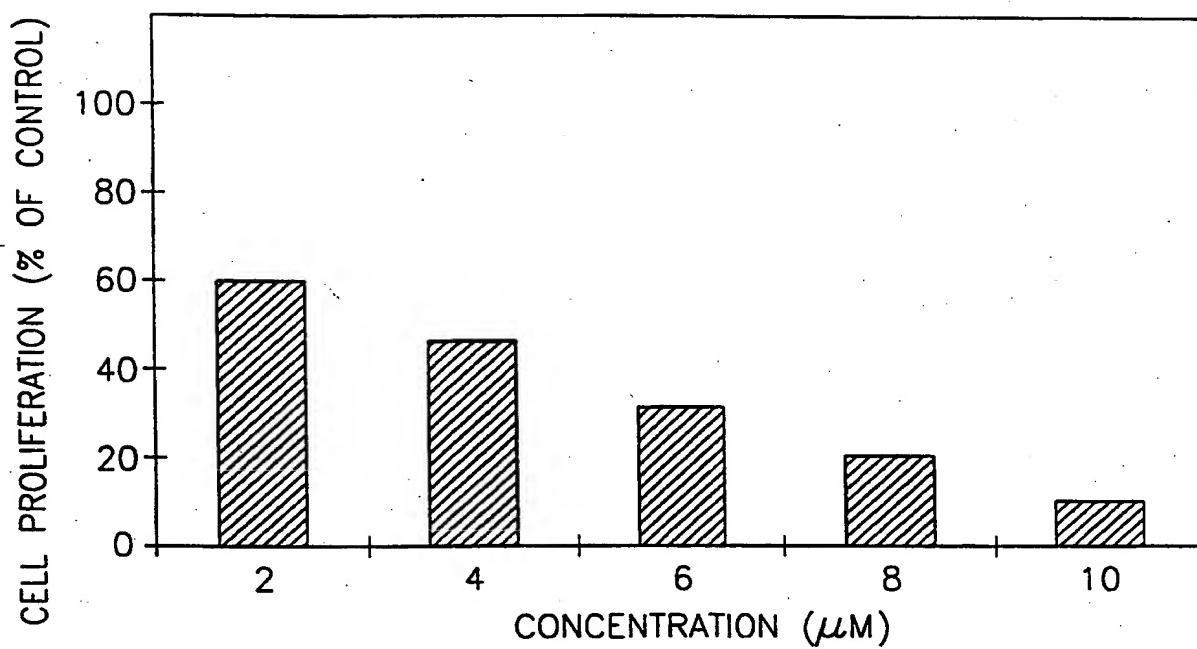


FIG.1

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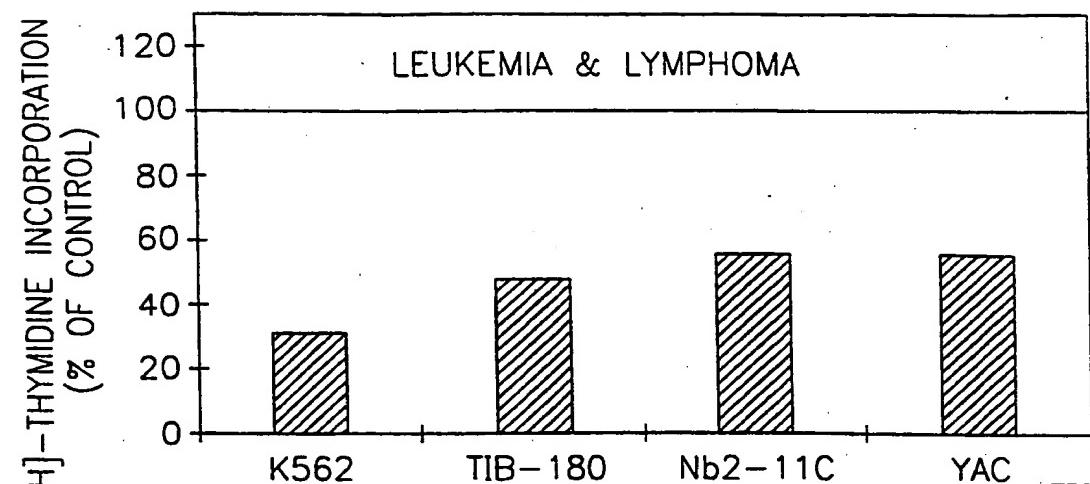


FIG.2A

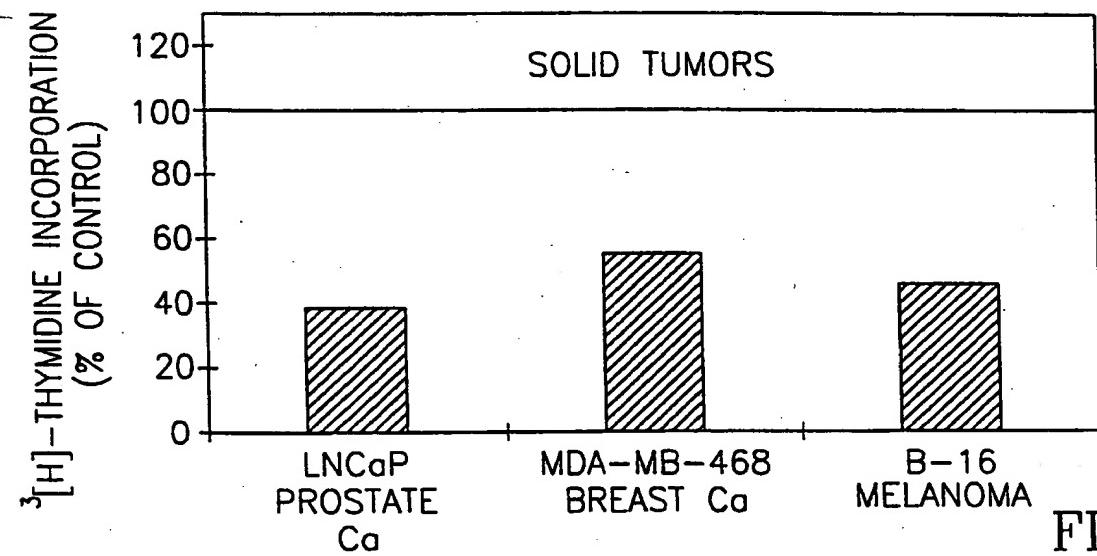


FIG.2B

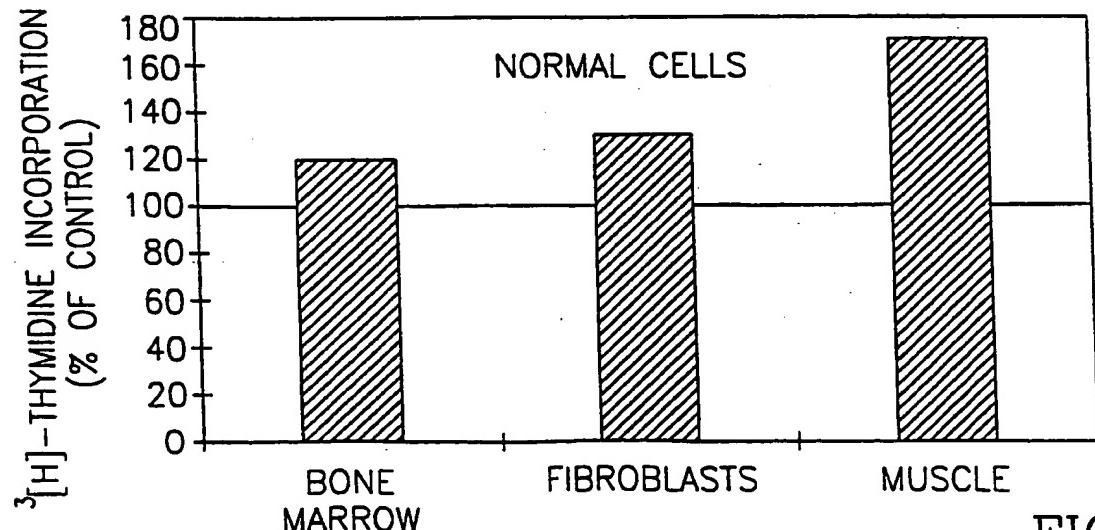


FIG.2C

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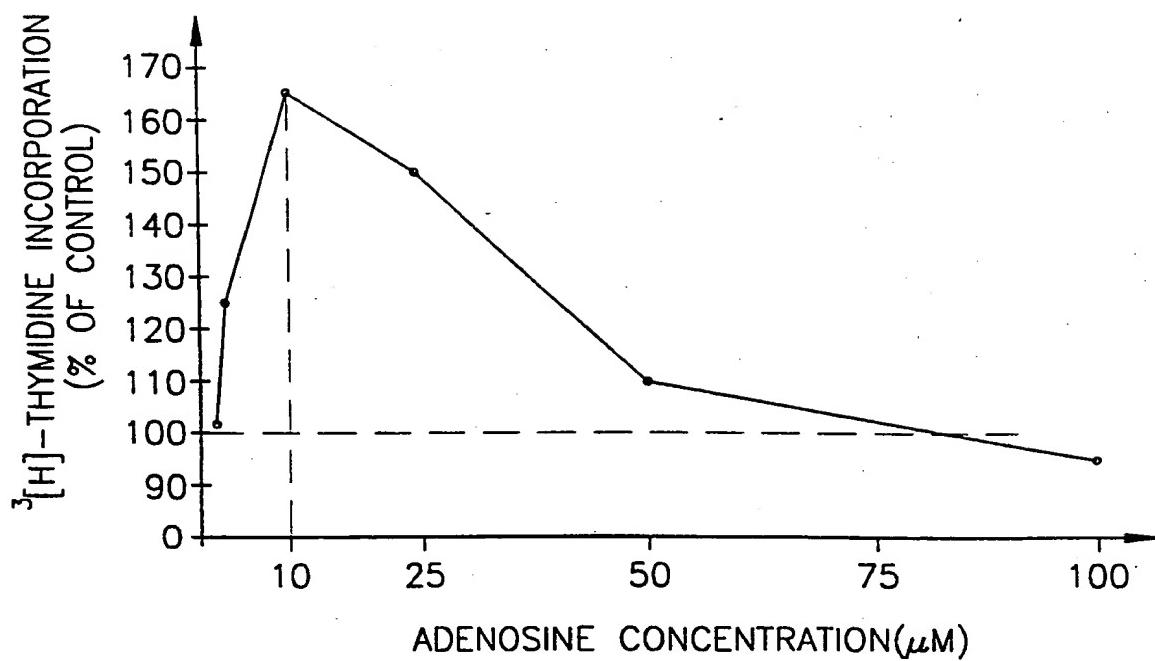


FIG.3

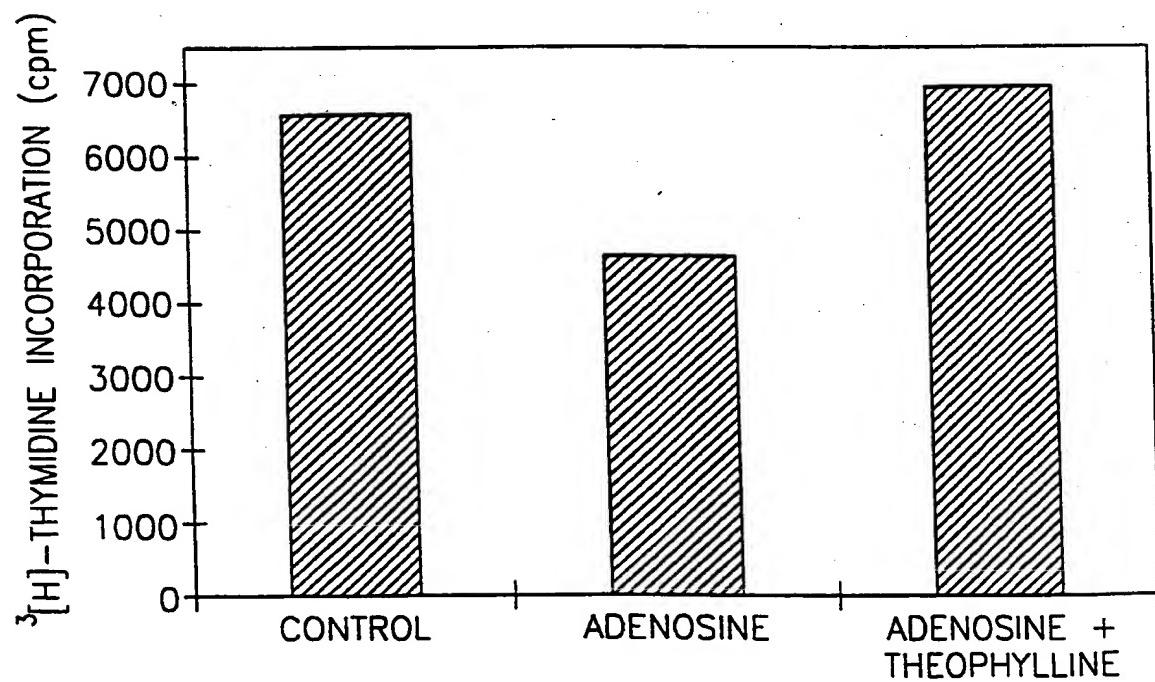


FIG.4

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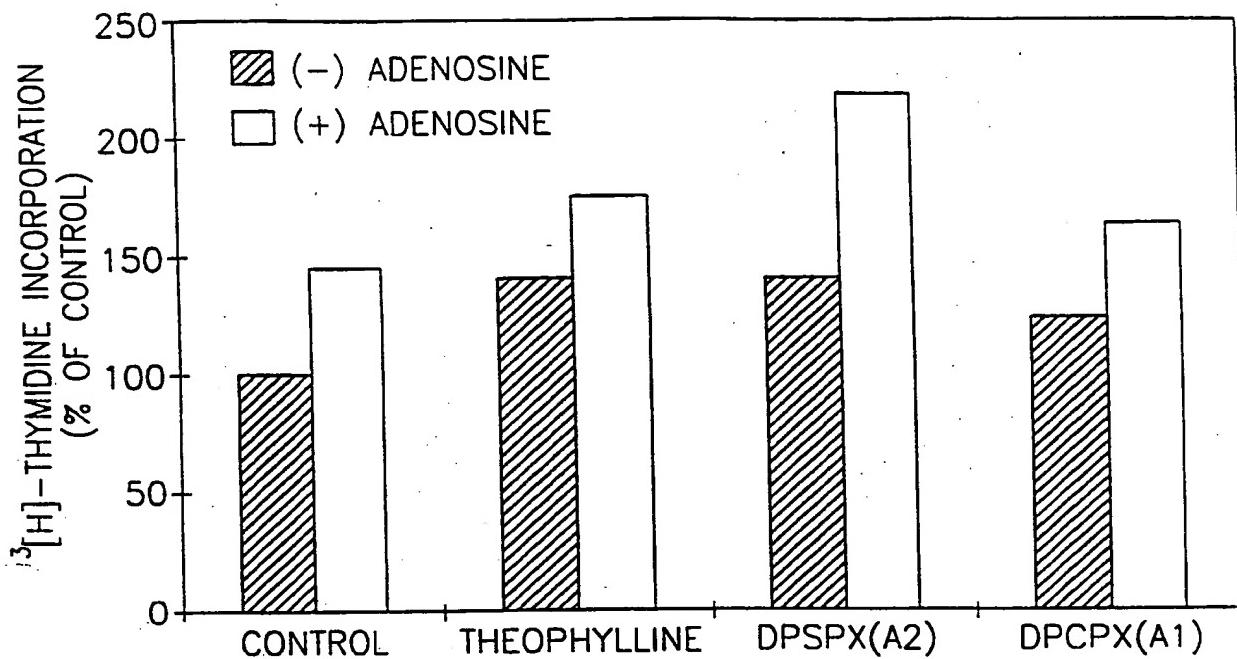


FIG.5

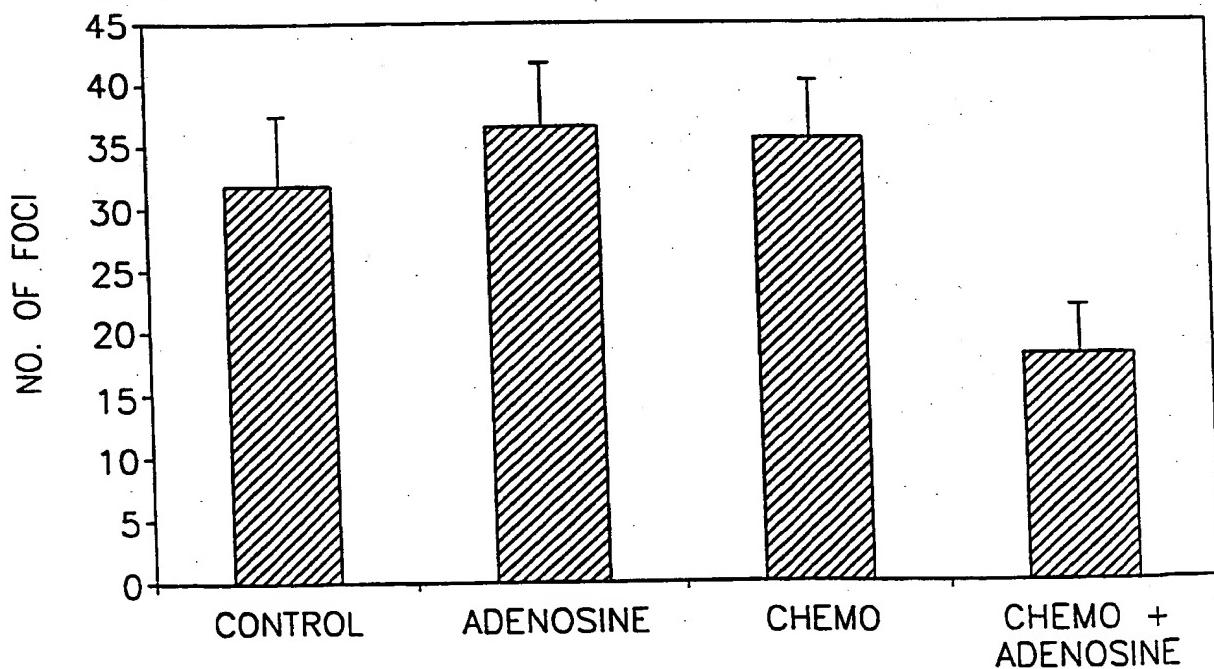


FIG.6

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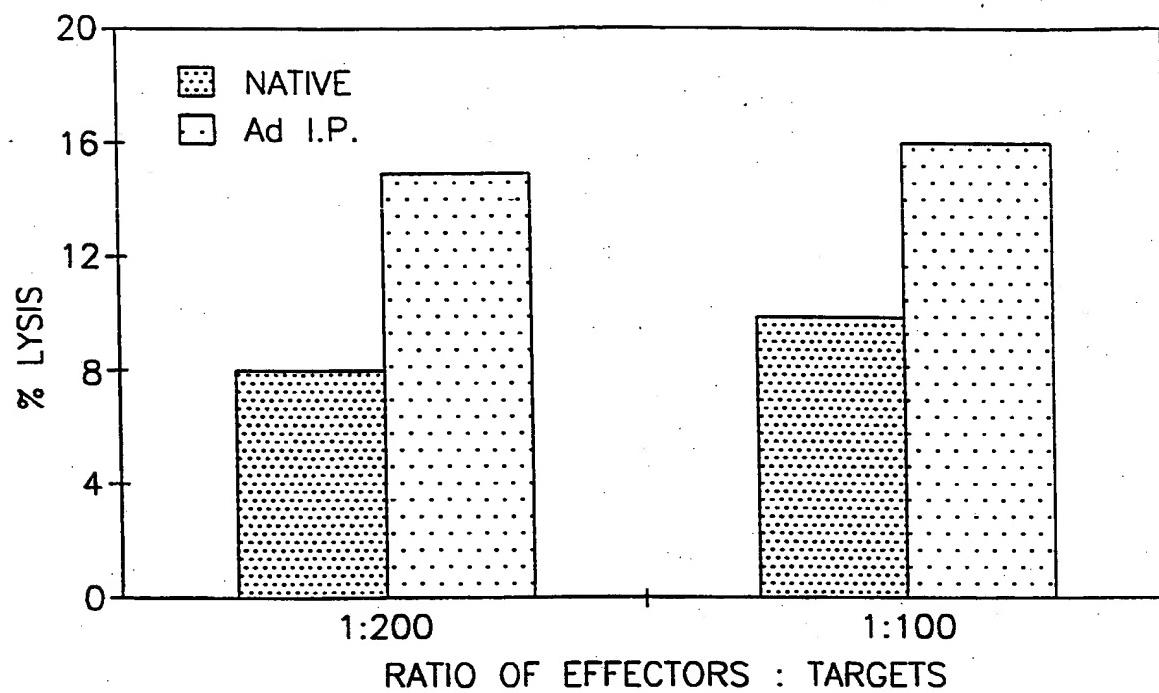


FIG.7

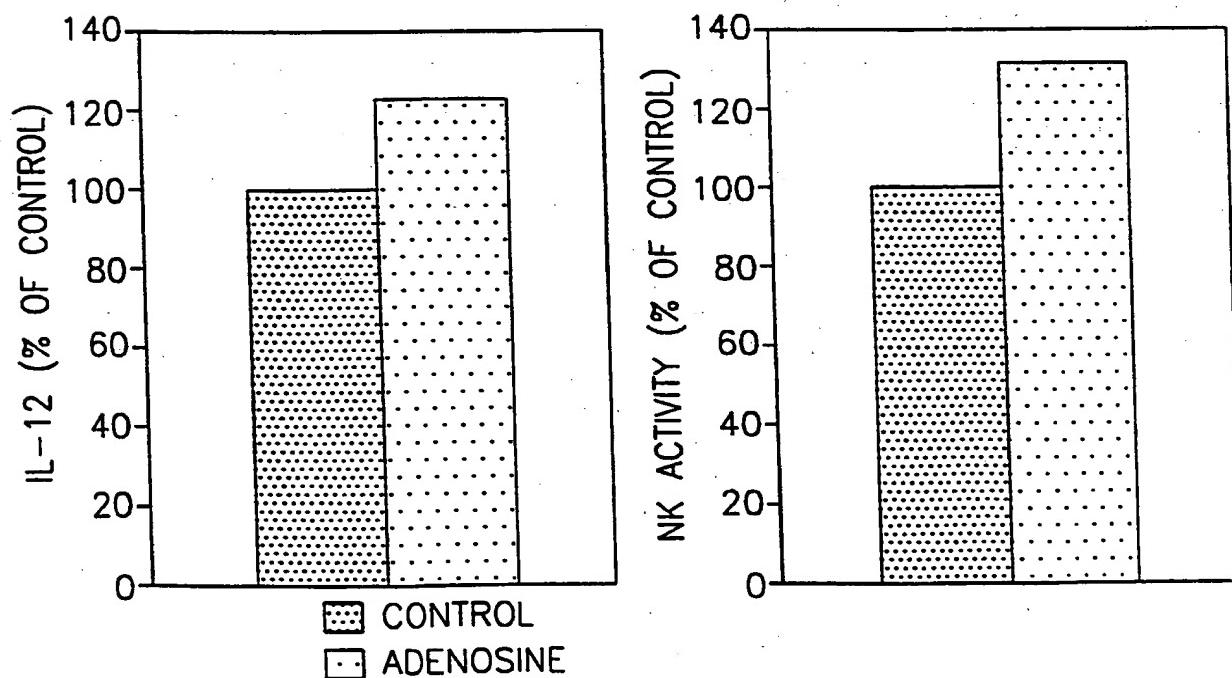


FIG.8



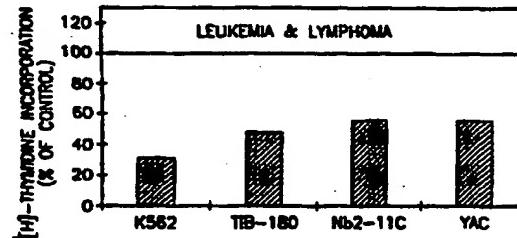
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(71) Applicant (for all designated States except US):	CAN-FITE TECHNOLOGIES LTD. [IL/IL]; Reinhold Cohn and Partners, P.O. Box 4060, 61040 Tel Aviv (IL).	
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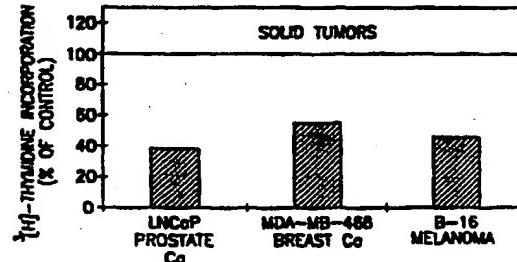
(54) Title: MEDICAMENT COMPRISING ADENOSINE

(57) Abstract

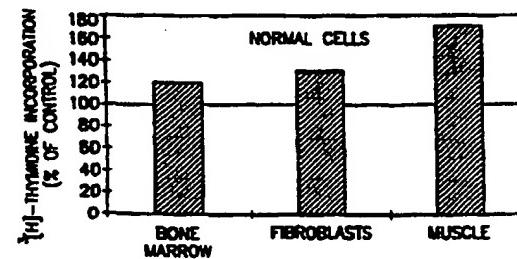
Adenosine and active agent which interact with the adenosine system are used to treat conditions of weakened, immune system, as an anti-cancer therapy and for improving the therapeutic index of a variety of therapeutic drugs.



A



B



C

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/IL 98/00324

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document* with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	I.BARASOAIN ET AL.: "ISOPRINOSINE RESTORES IN VITRO T LYMPHOCYTE FUNCTIONS OF CYCLOPHOSPHAMIDE IMMUNOSUPPRESSED MICE" INT. J. IMMUNOPHARMAC., vol. 9, no. 4, 1987, pages 489-496, XP002083810 see the whole document	1-5, 11-21, 26-32, 34,35
X	J.E.F.REYNOLDS, ED.: "MARTINDALE The Extra Pharmacopoeia, 31st Edition" 1996 , ROYAL PHARMACEUTICAL SOCIETY , LONDON XP002083816 see page 813 - page 814	1,6-10, 22-25, 33,35
		-/-



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"Z" document member of the same patent family

Date of the actual completion of the international search

6 May 1999

Date of mailing of the international search report

20.05.99

Name and mailing address of the ISA

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Authorized officer

Theuns, H

INTERNATIONAL SEARCH REPORT

Interr nal Application No

PCT/IL 98/00324

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	J. EPSTEIN ET AL.: "Protection of Normal Hematopoietic Stem Cells From the Toxicity of Purine Base Analogs: In Vivo Application" CANCER TREATMENT REPORTS, vol. 68, no. 9, September 1984, pages 1153-1156, XP002083811 cited in the application see the whole document ---	1-5, 11-21, 26-32, 34,35
X	RC.JACKSON ET AL.: "Biochemical Approaches to Enhancement of Antitumor drug selectivity: Selective Protection of Cells From 6-Thioguanine and 6-Mercaptopurine by Adenosine" CANCER TREATMENT REPORTS, vol. 64, no. 12, December 1980, pages 1347-1353, XP002083812 cited in the application see the whole document ---	1-5, 11-21, 26-32, 34,35
X	M. DEBATISSE ET AL.: "The Control of Cell Proliferation by Preformed Purines: A Genetic Study. II. Pleiotropic Manifestations and Mechanism of a Control Exerted by Adenylic Purines on PRPP Synthesis" SOMATIC CELL GENETICS, vol. 3, no. 5, September 1977, pages 513-527, XP002083813 see the whole document ---	1-5, 11-21, 26-32, 34,35
X	P.GENTILE ET AL.: "APPROACHES TO ABLATING THE MYELOTOXICITY OF CHEMOTHERAPY" CRC CRITICAL REVIEWS IN ONCOLOGY/HEMATOLOGY, vol. 7, 1987, pages 71-87, XP002083814 see page 73 - page 75 ---	1-5, 11-21, 26-32, 34,35
X	Y.-X. FENG ET AL.: "Suppressive effects of adenosine on nonspecific and humoral immunities in mice" DIALOG(R) FILE 73: EMBASE, ACCESSION NUMBER 9340892: ACTA PHARMACOL. SIN., vol. 15, no. 5, 1994, pages 473-476, XP002083815 see abstract ---	1-5, 11-21, 26-32, 34,35
		-/-

INTERNATIONAL SEARCH REPORT

International Application No

PCT/IL 98/00324

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	M.IIGO ET AL.: "Relationship between Antitumor Effect and Metabolites of 5-Fluorouracil in Combination Treatment with 5-Fluorouracil and Guanosine in Ascites Sarcoma 180 Tumor System" CANCER RES., vol. 43, no. 12 Pt. 1, December 1983, pages 5687-5694, XP002102063 see abstract ---	6-10, 22-25, 33,35
X	E. RAPAPORT: "Experimental Cancer Therapy in Mice by Adenine Nucleotides" EUR.J. CANCER CLIN. ONCOL., vol. 24, no. 9, 1988, pages 1491-1497, XP002102064 see the whole document ---	6-10, 22-25, 33,35
X	H.B. TEY ET AL.: "ADENOSINE MODULATES CELL GROWTH IN HUMAN EPIDERMOID CARCINOMA (A431) CELLS" BIOCHEM. BIOPHYS. RES. COMMUN., vol. 187, no. 3, 30 September 1992, pages 1486-1492, XP002102065 see the whole document ---	6-10, 22-25, 33,35
X	Q. WANG ET AL.: "Antineoplastic action of adenosine triphosphate" DIALOG (R) FILE 159: CANCERLIT (C): ACCESSION NUMBER 01001704: CHUNG HUA I HSUEH TSA CHIH, vol. 73, no. 7, 1993, pages 434-436, XP002102066 see abstract ---	6-10, 22-25, 33,35
X	E. RAPAPORT ET AL.: "Anticancer activities of adenine nucleotides in mice are mediated through expansion of erythrocyte ATP pools" PROC. NATL. ACAD. SCI. USA, vol. 86, March 1989, pages 1662-1666, XP002102067 see abstract ---	6-10, 22-25, 33,35
X	G.B. GRINDEY ET AL.: "Antitumor Activity of N6-Phenyladenosine, an Inhibitor of Adenosine Utilization, in Combination with Related Purine Analogs" CANCER RES., vol. 33, October 1973, pages 2459-2463, XP002102068 see abstract ---	6-10, 22-25, 33,35

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/IL 98/00324

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	M. TEZUKA ET AL.: "Potentiation of the Antitumor Effect of 5-Fluorouracil by Some Nucleotides, and their Possible Role in the Potentiation" JPN.J.EXP.MED., vol. 53, no. 3, June 1983, pages 155-163, XP002102069 see abstract ---	6-10, 22-25, 33,35
X	S. BAJAJ ET AL.: "Adenosine and Adenosine Analogues Are More Toxic to Chronic Lymphocytic Leukemia Than to Normal Lymphocytes" BLOOD, vol. 62, 1983, pages 75-80, XP002102070 see abstract ---	6-10, 22-25, 33,35
X	T.HIDAKA ET AL.: "EFFECTS OF A NEW ADENOSINE DEAMINASE INHIBITOR, ISOCOFORMYCIN, ON TOXICITY, ANTITUMOR ACTIVITY AND TISSUE DISTRIBUTION OF FORMYCIN A AND 9-BETA-D-ARABINOFURANOSYLADENINE" J. ANTIPIOT., vol. 33, no. 3, March 1980, pages 303-309, XP002102071 see page 303 ---	5-10, 22-25, 33,35
X	H. TANAKA ET AL.: "POTENTIATION OF CYTOTOXICITY AND ANTITUMOR ACTIVITY OF ADENOSINE ANALOGS BY THE ADENOSINE DEAMINASE INHIBITOR ADECYPENOL" J. ANTIPIOT., vol. 42, no. 11, November 1989, pages 1722-1724, XP002102072 see page 1722 ---	5-10, 22-25, 33,35
X	A.K. GHOSE ET AL.: "Structural mimicry of adenosine by the antitumor agents 4-methoxy- and 4-amino-8-(beta-D-ribofuranosylamino)pyrimido[5,4-d]pyrimidine as viewed by a molecular modeling method" PROC. NATL. ACAD. SCI. USA, vol. 86, November 1989, pages 8242-8246, XP002102073 see abstract -----	5-10, 22-25, 33,35

INTERNATIONAL SEARCH REPORT

b. International application No.
PCT/IL 98/00324

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 16-31 are directed to a method of treatment of the human/animal body, the search has been based on the alleged effects of the compound/composition.
2. Claims Nos.: 1, 3-6, 8-11, 13-16, 18-22, 24-26, 28-34 (all partially) because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
- No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-5, 11-21, 26-32, 34, and partially 35

Pharmaceutical composition for the treatment of a reduced level of leukocytes and for improving the therapeutic index of a drug having the side-effect of causing reduction in leukocyte levels.

2. Claims: 6-10, 22-25, 33, and partially 35

Pharmaceutical composition for anti-cancer treatment (as far as not comprised in the first group of inventions).

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Claims Nos.: 1, 3-6, 8-11, 13-16, 18-22, 24-26, 28-34 (all partially)

The definitions "an active agent other than adenosine selected from the group consisting of nucleosides, nucleoside derivatives and agents which interact with the adenosine system, said active agent being biologically active in inducing proliferation of bone marrow cells", resp. "an active agent other than adenosine selected from the group consisting of nucleosides, nucleoside derivatives and agents active in the adenosine system, said active agent being biologically active in inhibiting proliferation of tumor cells", resp. "..., said active agent being biologically active in either increasing efficacy of the therapeutic drug or inducing proliferation of bone marrow cells" do not represent full definitions of chemical compounds by means of structural features. In view of the definitions by means of pharmacological parameters and the fact that the "desiderata" expressed in these definitions are drafted as to contain pointers to the solutions claimed, a meaningful search is virtually impossible. The search was limited to pharmaceutical compositions comprising adenosine.

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